Supplementary Figures

Supplementary Figure 1. Injection of liver purified iNKT cells, depleted of iNKT cells, failed to increase the survival of PR8 infected Jα18$^{-/-}$ mice. WT and *Jα18*$^{-/-}$ mice (n=5/ group) were injected i.n. with PR8. Liver purified iNKT cells were simultaneously stained with anti CD5, anti TCRβ specific antibodies and CD1d/α-GalCer tetramers and triple positive cells, representing 75% of the total population, were removed. Mice were injected 1 day post infection either with the total population sorted with anti CD5 and anti TCRβ specific antibodies (CD5$^+$ TCRβ$^+$ sorted cells) or with CD5$^+$ TCRβ$^+$ cells from which the CD1d/α-GalCer tetramer$^+$ cells were depleted (CD5$^+$ TCRβ$^+$ sorted cells depleted of iNKT cells). Survival rate is shown as the percentage of live mice at different time points after the infection.

Supplementary Figure 2. Phenotypic and functional analysis of MDSC.

(**A**) Phenotypic analysis of BM derived MDSC (gated on Gr-1$^+$ and CD11b$^+$ cells) stained with CD40, CD1d and TLR9 antibodies (open histograms). Grey histograms indicate no antibody staining.

(**B**) MDSC suppress CFSE labelled OT-I proliferation induced by matured DC. DC from BM culture were matured with LPS (10μg/ml) or α-GalCer in the presence of iNKT cells and then pulsed with SIINFEKL peptide for 2 h. OT-I splenocytes were cultured with matured DC ($5x10^4$) and in the presence or absence of MDSC ($3x10^4$). CFSE labelled OT-I splenocytes alone are shown in green histograms.

(**C**) Staining with CD1d/α-GalCer tetramers and anti CD3 antibody of iNKT cells in CD11c and Gr-1 depleted BM cultures.

Supplementary Figure 3. Lack of iNKT cell dependent lysis of α-GalCer pulsed MDSC.

CFSE labelled BM derived MDSC were either pulsed or not pulsed with α–GalCer and incubated with CD11c and Gr-1 depleted BM cultures, which contain iNKT cells. The percentage of Bisbenzimide negative CFSE labelled MDSC is very similar in the α–GalCer pulsed and unpulsed cultures, indicating that MDSC pulsed with 100 ng of α–GalCer were not sensitive to killing by iNKT cells. As a positive control for iNKT cell activity, the right panel shows CD11c expression on CFSE positive and Bisbenzimide negative MDSC, 48 h after incubation with iNKT cells. Blue histogram shows CD11c expression on α-GalCer pulsed CFSE labelled MDSC in the presence of iNKT cells, while green histogram shows CD11c expression on unpulsed CFSE labelled MDSC in the presence of iNKT cells. Red histogram shows CD11c expression on unpulsed CFSE labelled MDSC in absence of iNKT.

Supplementary Figure 4. MDSC can be infected by PR8 virus.

(**A**) MDSC from lungs of WT, *Jα18*$^{-/-}$ and *CD1d*$^{-/-}$ mice infected i.n. with PR8 ($3x10^4$ pfu/10^6 cells) were purified with anti-CD11b-coated magnetic beads. The cells were analysed using semi-quantitative PCR.

(**B**) BM derived MDSC from WT mice were infected with PR8 ($2.5x10^4$ pfu/10^6 cells) for 1 h. Cells were analysed for expression of PR8 nucleoprotein (NP) 12 hrs and 24 hrs later using semi-quantitative PCR.

Supplementary Figure 5. Phenotypic changes of CpG treated MDSC.

(**A**) CpG can induce IL-12p40 secretion from *hexβ*$^{-/-}$ and *iGb3S*$^{-/-}$ MDSC in the presence or absence of anti CD1d antibody. Supernatants were harvested at 48 h.

(**B**) MDSC derived from BM of *hexβ*$^{-/-}$, *hexβ*$^{-/+}$, *iGb3S*$^{-/-}$ and *iGb3S*$^{-/-}$ mice were treated with CpG for 24 h. Treated and untreated MDSC were stained with anti CD1d-PE antibody.

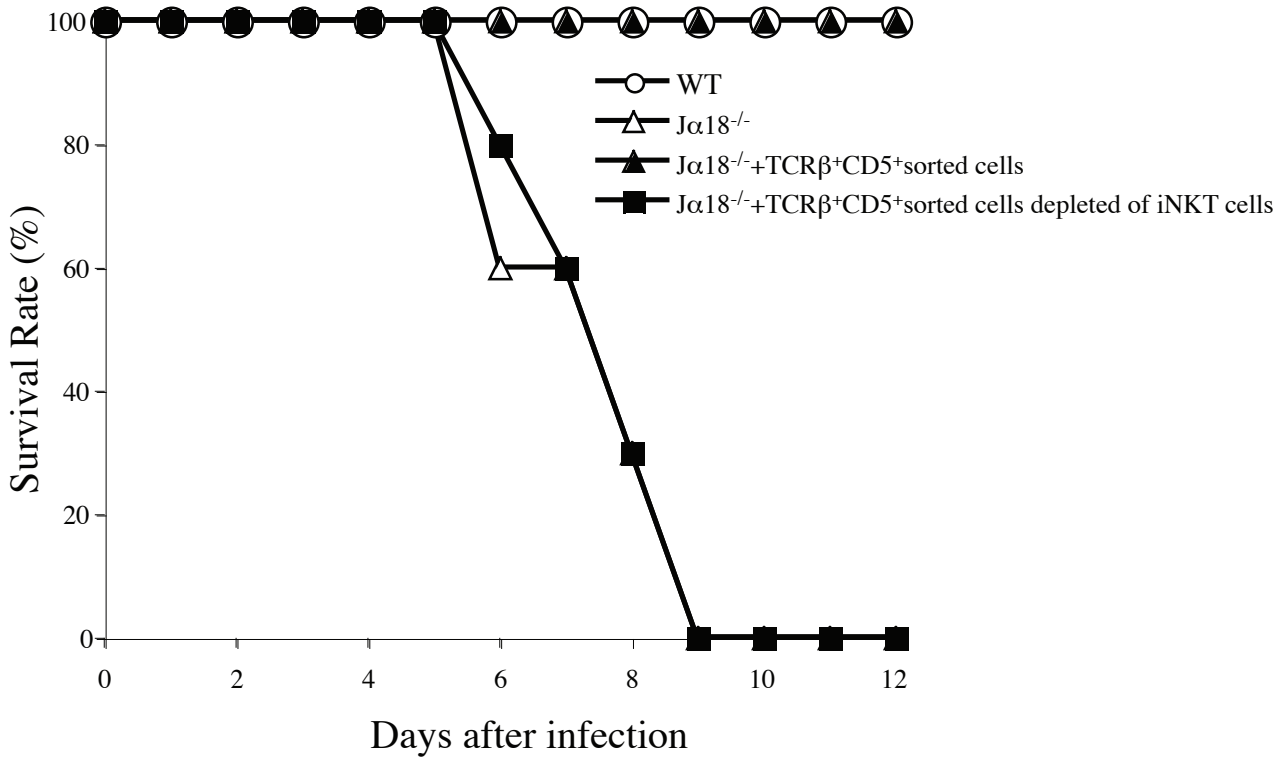
Supplementary Figure 6. **H17 IAV (H3N2) infection induces a greater expansion of MDSC in Jα18$^{-/-}$ mouse than in WT mice.** Expansion of lung infiltrating CD11b^{+} Gr-1^{+} cells in *Jα18*$^{-/-}$ and in WT mice injected i.n. with H17 IAV (20 HAU), which peaked at approximately day 9 after the infection. Data represents the average of n= 5 mice/ group ± S.D.

Supplementary Figure 7. **Inhibition of T cell proliferation by human MDSC can be rescued by iNKT cells.**

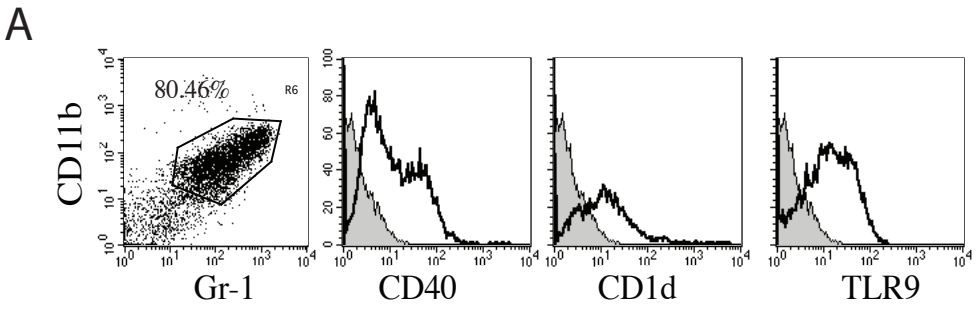
(**A**) CD11b^{+} cells, purified from PBL either from individuals shortly after an acute respiratory illness (associated with the presence of high IAV specific Ab titer shown in Table 1) or from healthy donors were added to PBL and incubated with allogeneic irradiated DC. Purified CD11b^{+} cells were either untreated (■), or treated with either α-GalCer (100 ng/ml) in presence of iNKT at a MDSC:iNKT cell ratio of 1:0.25 (□) or L-NMMA and NOHA (▣). The data are expressed as described in Methods. Addition of either iNKT cells or L-NMMA and NOHA to the alloreactive T cells in the absence of CD11b^{+} cells did not affect T cell proliferation (data not shown). The ratio of irradiated DC to purified irradiated CD11b^{+} cells was 1:1.

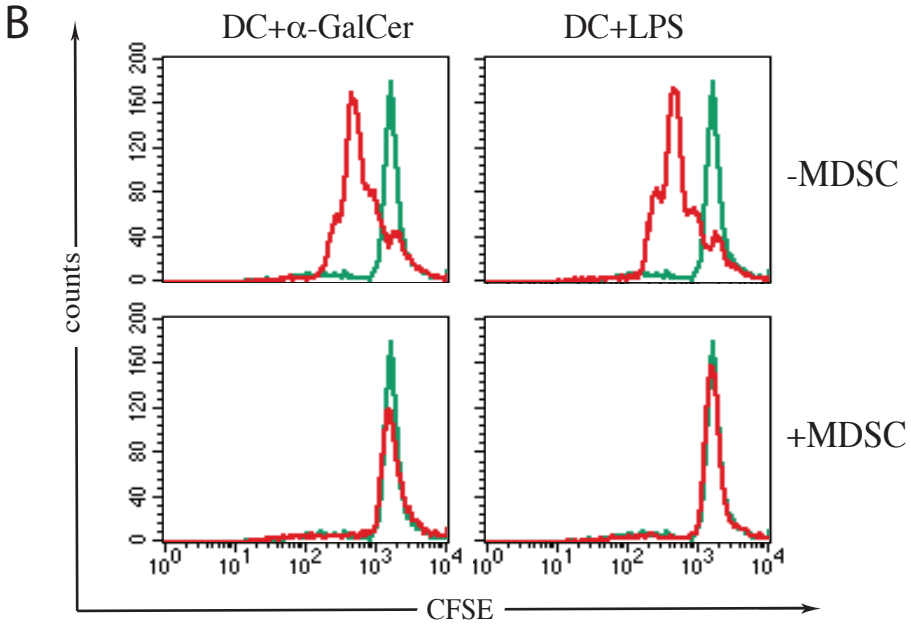
(**B**) Conditions to prevent lysis of α-GalCer pulsed human MDSC by iNKT cells. Human MDSC ($0.5x10^6$) were treated with α–GalCer (100 ng/ml) and co-cultured with increasing numbers of iNKT cells for 24 h. MDSC were stained with Propidium Iodide to calculate the percentage of iNKT cell dependent MDSC killing.

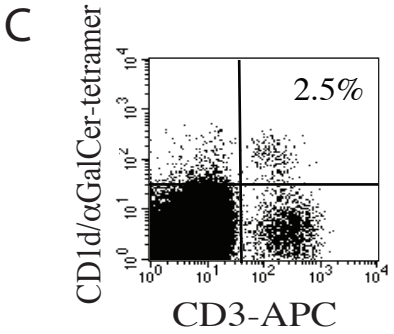
(**C**) iNKT cells are activated by α-GalCer pulsed $CD11b^+$ cells from IAV infected patients. IFN-γ release from iNKT cells incubated for 24 h with α-GalCer pulsed $CD11b^+$ cells, derived from the IAV infected patients and healthy controls described in Figure 7. Data represent the average of 3 replicates ± S.D.



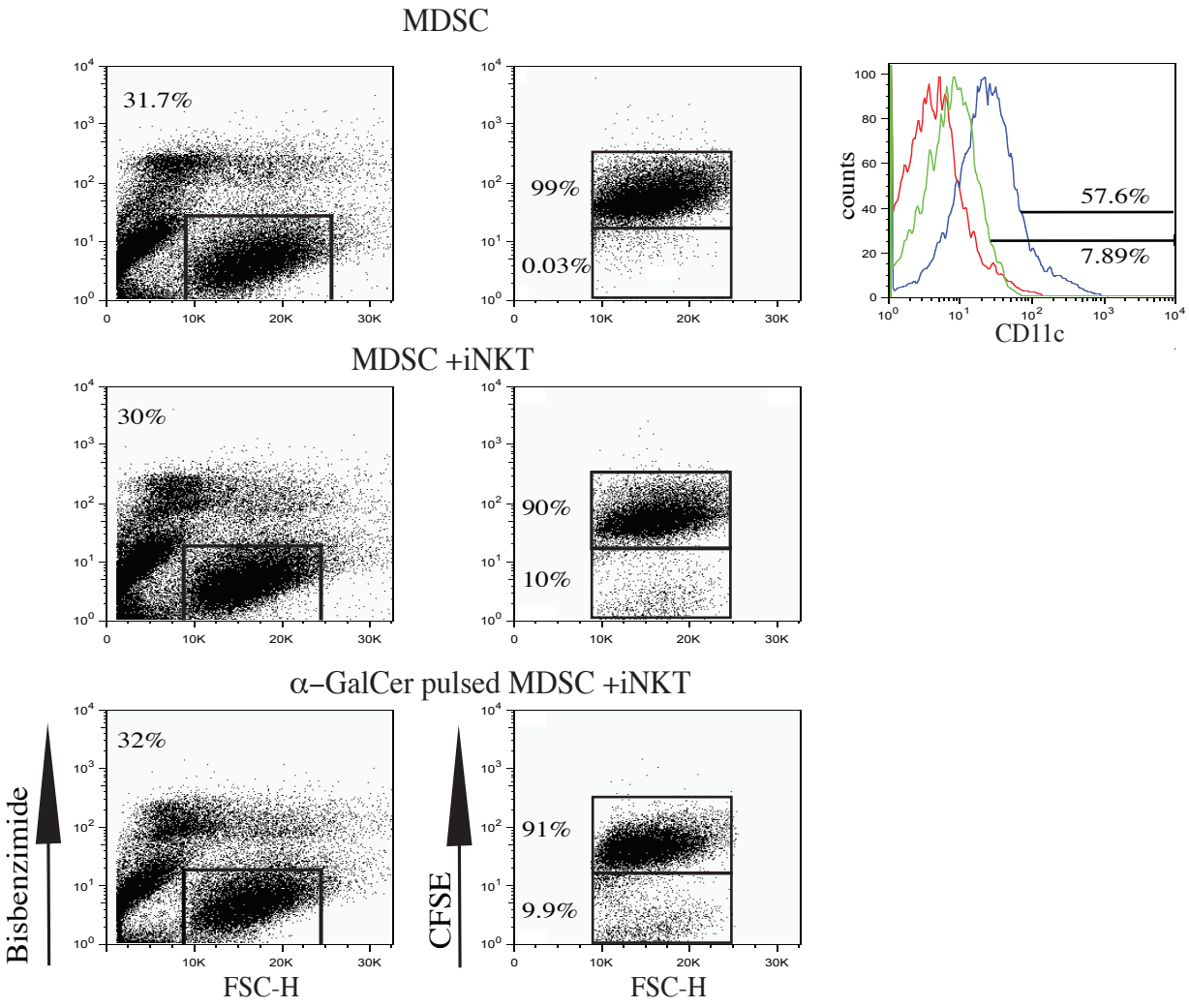
Supplementary Figure 1





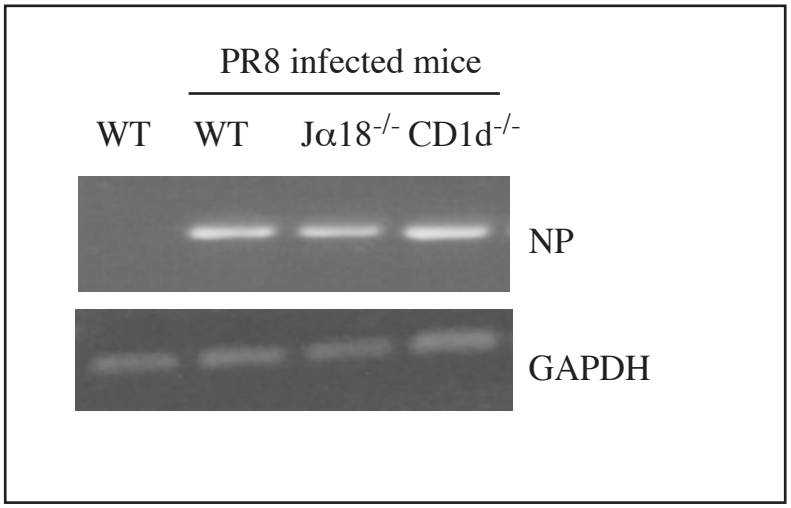


Supplementary Figure 2



Supplementary Figure 3

A



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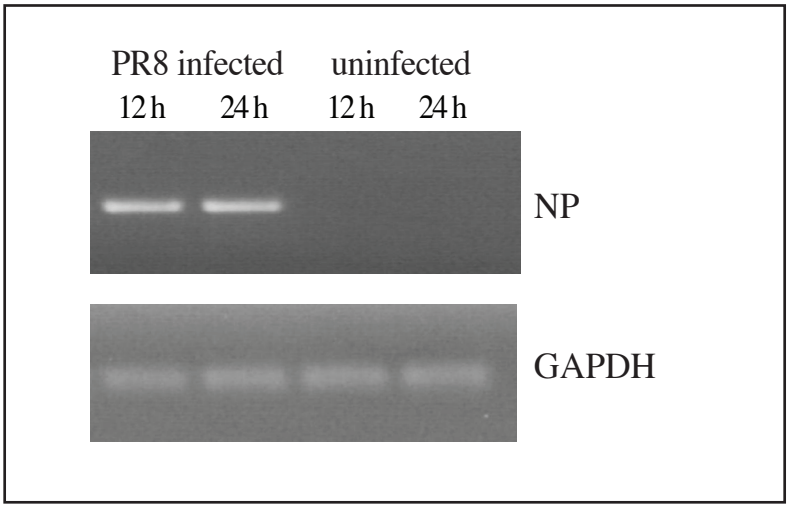
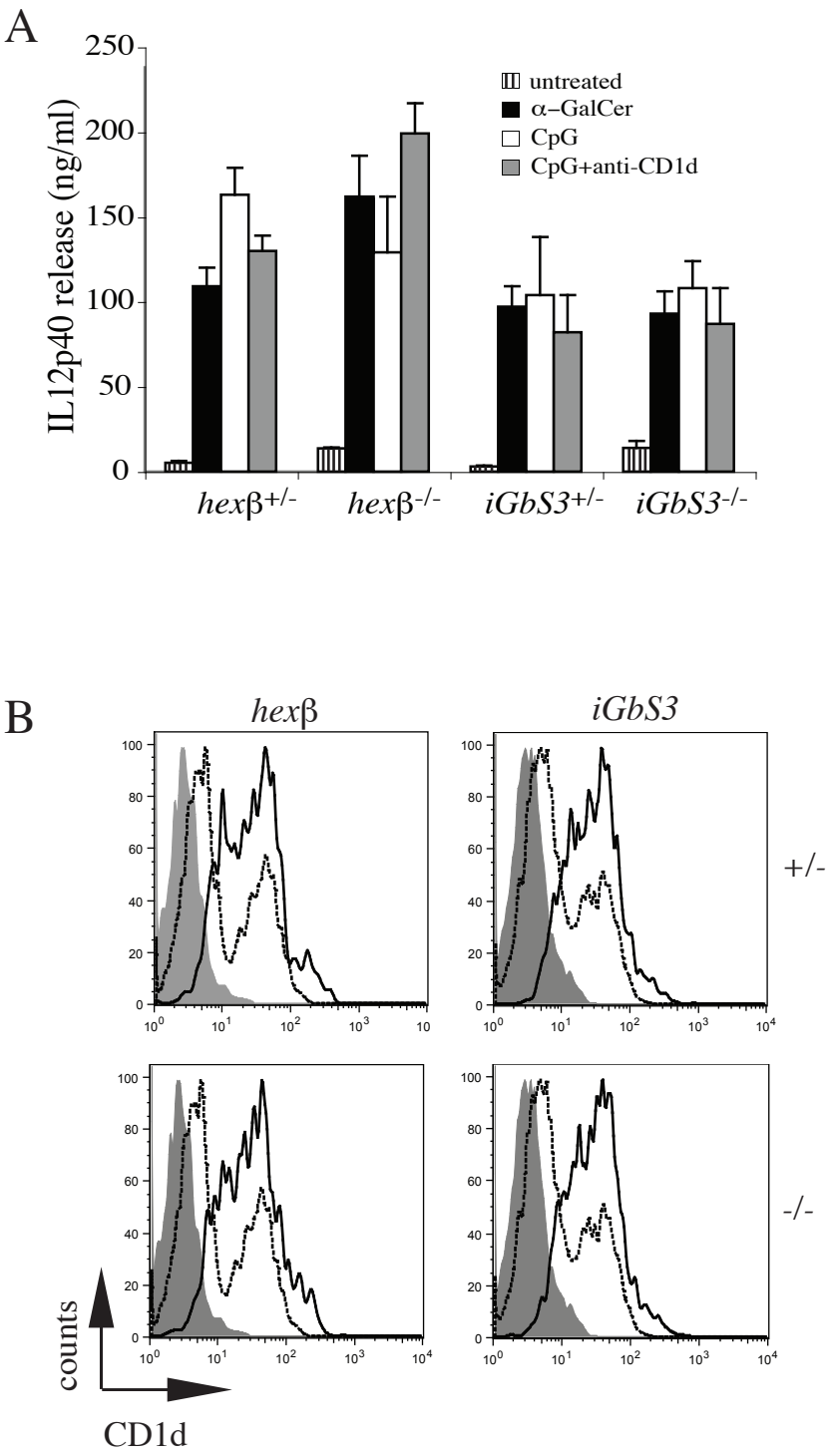
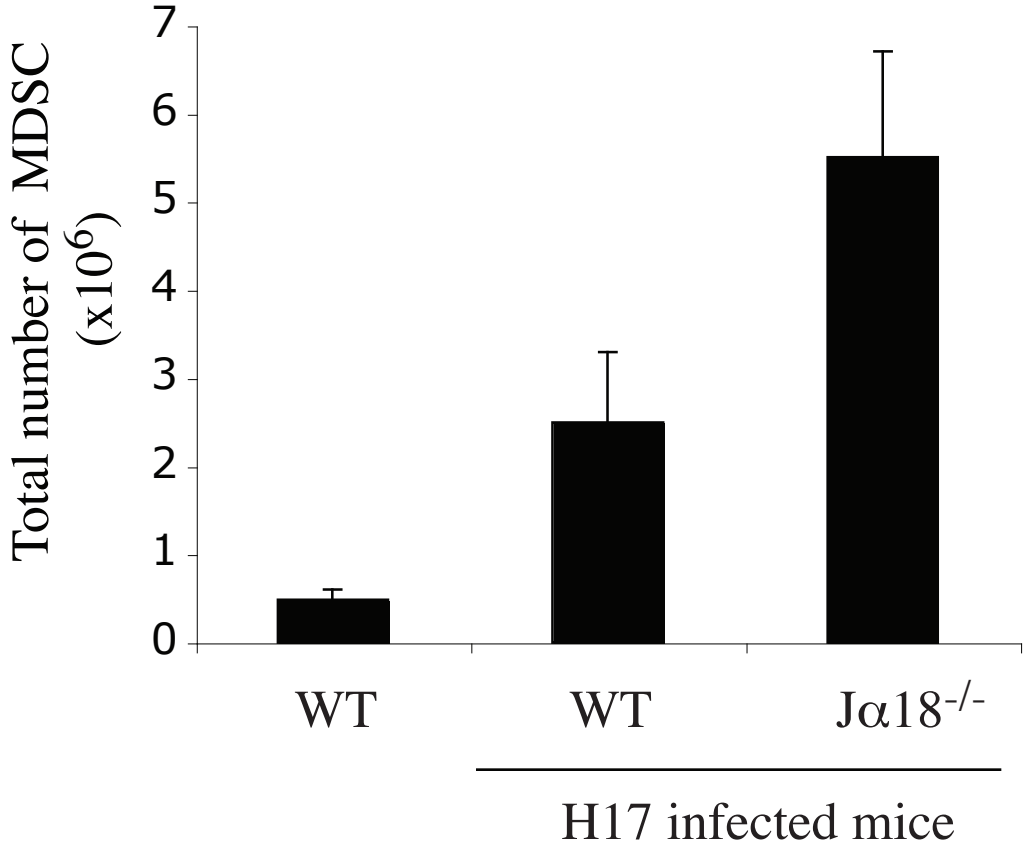


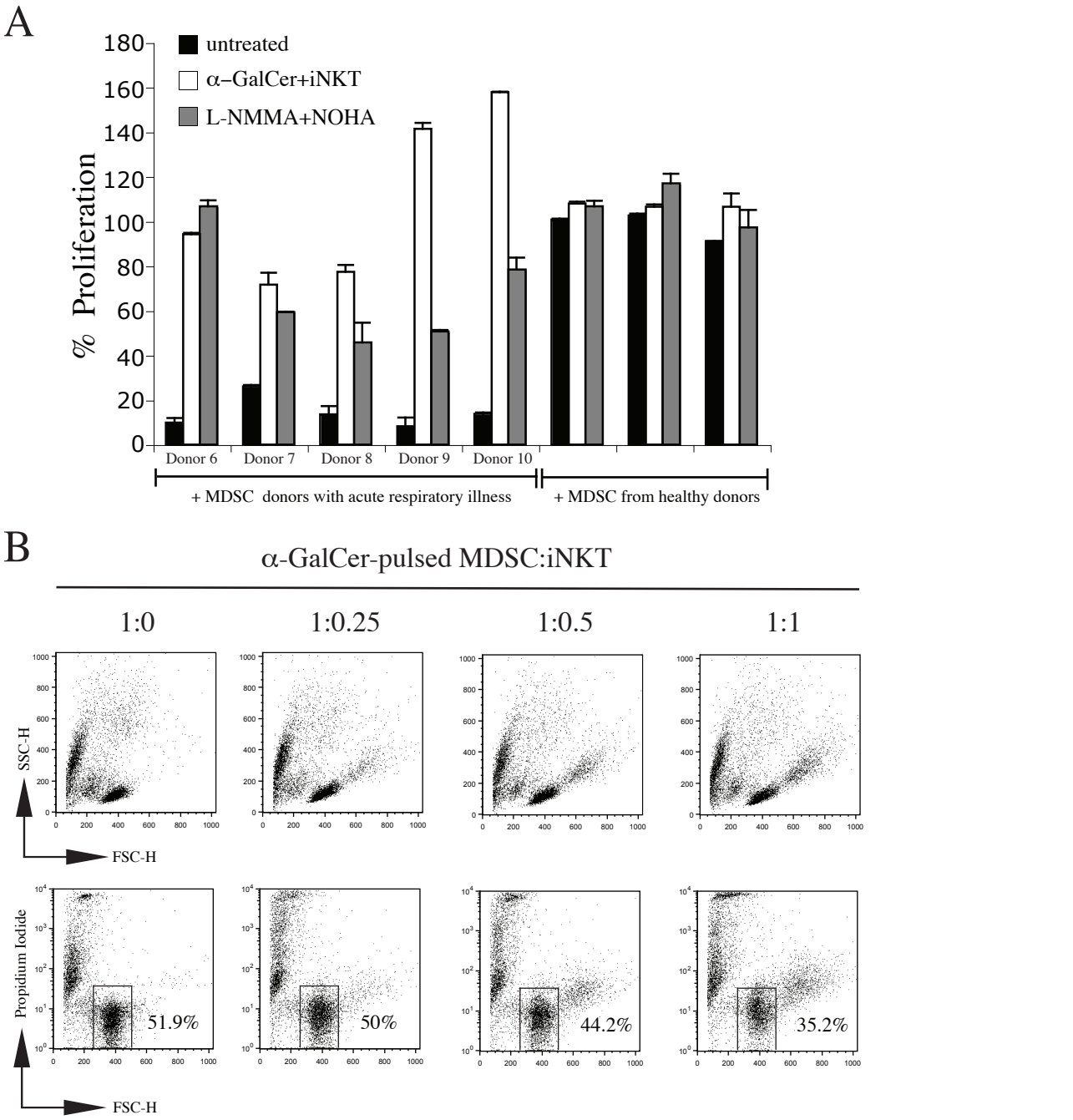
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7